REMARKS

Claims 114-168 are pending in the application. The amendment to claim 157 merely corrects a typographical error. The title is amended to more clearly reflect the claimed invention. No new matter has been added.

All of the claims were rejected.

The Invention

The present invention is drawn to methods of providing a therapeutic product to a mammal. The therapeutic product is produced by cells in which an endogenous gene encoding the protein is activated so that the protein is expressed from the genome of the cell. Activation in accordance with the invention is an *in vitro* process that involves targeted homologous recombination to place an exogenous regulatory sequence at a selected site within the genome in order to activate a selected endogenous gene. The construct used to accomplish this includes not only the exogenous regulatory sequence, but also (1) a targeting sequence, (2) an exon, (3) a splice-donor site, (4) an intron, and (5) a splice-acceptor site. The construct is introduced into a targeted cell *in vitro* by transfection (i.e., using a nonviral vector), and then homologously recombines with the cell's genome at a target site in the genome of the targeted cell. Transcription produces a transcript that contains sequence corresponding to the construct-derived exon, the construct-derived splice-donor site, the construct-derived intron, the construct-derived splice-acceptor site, and all of the exons of the endogenous gene, such that the RNA transcript encodes the therapeutic product. When this transcript undergoes splicing, the construct-derived splice-donor site interacts with the construct-derived splice-acceptor site. This results in the splicing out of the construct-derived intron. Because this entire process is carried out *in vitro*, the resulting homologously recombined cells can be cultured. Those expressing the desired level of protein can be selected, expanded, and characterized prior to implantation of the cells into the mammal. This *ex vivo* method is therefore quite different from, and eliminates many of the uncertainties of, *in vivo* gene therapy techniques.

35 U.S.C. § 112, First Paragraph

All of the pending claims are rejected for alleged lack of enablement. Applicants respectfully traverse the rejection.

The Examiner has listed the factors that were considered in the objection to the specification and the associated rejection of the claims. These factors are essentially the so-called Wands factors of *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988), used to determine whether a disclosure would require undue experimentation. We shall address each of those factors separately.

The nature of the invention

The Examiner states that the invention "is drawn to a gene therapy method, particularly employing homologous recombination, i.e., "gene targeting", either in vivo or ex vivo" (Office Action at page 1). Applicants agree with the examiner that the invention involves an *ex vivo* method, but do not agree that the invention involves an *in vivo* method. Claim 1, the sole independent claim, specifically states that the cell is "generated by an *in vitro* process." Requiring that the transfection process take place *in vitro* precludes characterization of the claim as covering *in vivo* gene therapy.

Applicants further point out that, in specifying that the construct is "transfected" into the cell, the claims exclude virally-mediated gene therapy. There are four basic categories into which the field of gene therapy can be divided: *in vivo*/viral, *in vivo*/nonviral, *ex vivo*/viral, and *ex vivo*/nonviral. Only the fourth category encompasses the present invention.

Applicants respectfully submit that, although the Office Action acknowledges that the present application is drawn to *ex vivo* methods, no aspect of the rejection for lack of enablement appears to be relevant to *ex vivo* methods. As elaborated below, the rationale behind the rejection is pertinent solely to *in vivo* methods of gene therapy and therefore does not apply to the claimed invention.

The amount of direction or guidance presented in the specification, and the presence or absence of working examples

The Office Action states that "Applicants present general guidance in the form of short lists of moieties which could exhibit targeting of the complexes to certain organs, or at least groups of cells" (Office Action at bridge of pages 1-2). This statement does not appear to be relevant to the present claims. First, Applicants are unsure what the Examiner means by "the complexes," a term that does not appear in the claims and seems unrelated to the present invention. Second, Applicants do not target anything (complexes to otherwise) to organs or "groups of cells" (the transfection being done by an *ex vivo* method that does not require such targeting). Finally, the statement about "lists of moieties which could exhibit targeting" appears to bear no relevance to the present invention. The only "targeting" pertinent to the claim invention relates to homologous recombination of DNA using a "targeting sequence," i.e, a nucleic acid sequence that can homologously recombine at the appropriate place in genomic DNA. The Examiner does not seem to be challenging enablement on the basis of lack of guidance concerning homolgous recombination (and indeed should not, as it is routinely accomplished by those of ordinary skill in the art), so it is unclear what issue is being raised here. Clarification is respectfully requested.

The Office Action also states that "[a]pplicants give limited guidance for the selection of dosages, but these are not in view of any particular nucleic acid construct or genetic defect/disease." (Office Action at page 2). Thus, the Office Action appears to require that the specification provide specific information about selection of dosages for specific diseases and constructs.

Because of the nature of the present invention, the particular construct utilized in any given case is not what dictates dosage. Dosage (in terms of the number of cells introduced into the mammal) is dictated by the amount of therapeutic product produced by each cell, a determination that can be made by measuring the output of a defined number of transfected cells in culture. This is, of course, a simple and routine task. If a particular construct produces transfected cells that secrete a relatively low amount of the protein, a proportionally higher number of cells could be implanted into the mammal. Accordingly, giving "guidance" about dosage with respect to any specific construct is unnecessary and superfluous.

Nor should it be necessary to describe the dose of cells to use for any given disease. The prior art contains numerous examples of protein therapies, including dosages of the protein, to alleviate various disorders. To name two examples, the use of glucocerebrosidase for treatment of Gaucher's disease is well documented, as is the use of growth hormone to treat growth hormone deficiency. A number of proteins that can be delivered in accordance with the invention are described in the references enclosed in the Supplemental Information Disclosure Statement submitted herewith. These include interferons (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1190-1191), tissue plasminogen activator (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1323-1324), calcitonin (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, p. 1509), colony stimulating factors (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1281-1282); growth hormone (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, p. 1342), glucocerebrosidase (Harrison's Principles of Internal Medicine, 12th ed., Eds. J.D. Wilson et al., McGraw-Hill, Inc., NY, 1991, p. 43), and Factor IX (Harrison's Principles of Internal Medicine, 12th ed., Eds. J.D. Wilson et al., McGraw-Hill, Inc., NY, 1991, p. 1507). The known methods for administering these proteins include systemic and local administration, either of which is possible using the methods of the invention. Doses of the proteins effective for achieving a positive therapeutic outcome with each of the stated conditions are also known. As the Examiner is well aware, it is not necessary to set forth in the specification that which is already known in the art.

Once homologously recombined cells producing a given protein have been generated, selected, expanded, and characterized, the approximate number of cells needed to deliver the desired dose of protein is calculated and implanted in the patient, for example, in a barrier device (e.g., as discussed in the specification of U.S.S.N. 07/985,586 at page 35, line 27-page 36, line 23. The present application claims priority to and incorporates 07/985,586 by reference). The number of cells implanted can then be adjusted up or down as necessary to produce the desired clinical effect. This kind of adjustment of dosage is routine for any pharmaceutical

agent, and certainly does not constitute undue experimentation, particularly in view of the knowledge in the art regarding the use of proteins for treatment of disease.

It would not have been feasible for Applicants to provide specific treatment regimes for every conceivable disorder that can be treated using the present invention. There is no reason to expect the treatment protocols to be complicated or involve extensive experimentation, since methods for successfully implanting cells are known. The invention is a method of delivering a protein using cells that express the protein. Essentially any protein encoded by a gene endogenous to the cells can be delivered by this method. An illustrative analogy may be drawn to the use of a new type of syringe to deliver a therapeutic substance. Such a syringe would not be considered nonenabled just because a list of substances, treatable conditions, protocols, dosages, and therapeutic outcomes using the syringe was not provided. Likewise, it should not be required in the present case.

The state of the prior art.

The Office Action cites Orkin et al. as evidence that

> clinical efficacy has not been definitively demonstrated at [that] time in any gene therapy protocol,' that '[s]ignificant problems remain in all basic aspects of gene therapy,' and that available vectors, and understanding in the art of the interactions between said vectors with the host, are inadequate. (Office Action at page 2)

Orkin et al. is the "Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy." The Office Action's characterization of Orkin may be accurate with respect to viral vectors, and with respect to *in vivo* gene transfer in general, but a careful reading of Orkin et al. discloses no such statements relevant to the present invention (which involves neither viral vectors nor *in vivo* gene transfer). Table 1 of Orkin et al. notes as the only drawbacks to "naked DNA" and "facilitated DNA" (the systems most relevant to the nonviral vectors of the present invention), that they exhibit, respectively: "very inefficient entry, uptake into nucleus. No mechanism for persistence or stability" and "targeting not yet achieved. No mechanism for persistence or stability. Inefficient entry." These are potential problems if *in*

vivo gene transfer is what is being attempted. Because the claimed invention utilizes *in vitro* gene transfer to activate an endogenous gene followed by cloning and selection of successfully transfected cells, the cited problems of lack of targeting to specific cells, inefficient uptake, and inadequate persistence of the vector are simply not an issue.

The Office Action also cites Anderson as making it

> clear that methods extant in the art, particularly vector selection, delivery methods and persistence of gene expression, were still inadequate to permit routine practice of gene therapy, let alone any demonstrably successful practice at all. (Office Action at page 2)

None of these issues is pertinent to the present invention. Vector selection and delivery methods are problems in *in vivo* gene therapy, but not *ex vivo*, where transfection efficiency can be boosted by techniques such as electroporation, and where even low efficiency can be tolerated because the cells can be selected and expanded prior to use. Persistence of gene expression is not expected to be an issue because the exogenous DNA is homologoulsy recombined into the genome in a defined site, and stably transfected cells exhibiting satisfactory gene expression over many generations can be selectively cloned and used.

The Office Action cites the first paragraph and the concluding paragraphs of Anderson as further evidence that gene therapy was not routine in 1998. Applicants note that these passages both emphasize low efficiency of gene transfer and expression as the critical problem. As pointed out above, these are simply not issues in the presently claimed methods.

Applicants submit that neither the Orkin nor the Anderson reference raises enablement issues relevant to the present invention. As the only prior art cited in the Office Action as describing the "State of the prior art," they do not support the Examiner's grounds for rejection of the present claims.

Predictability or unpredictability of the art.

The Office Action cites three references to illustrate the alleged unpredictability of the prior art: Orkin et al., an editorial from Nature Biotechnology ("the editorial"), and Verma et al.

Orkin is specifically cited for its admonition that animal models may not always "satisfactorily mimic the major manifestations of the corresponding human disease." The relevance of focusing on this aspect of Orkin is not clear to the Applicants. Orkin was referring to animal models of human genetic diseases, while the present invention is merely a new method of delivering a protein. The animals utilized for *in vivo* tests disclosed in the specification were normal animals, not animal models of human genetic diseases. They were not intended to display symptoms of a human disease, but rather to demonstrate that cells manipulated *in vitro* so that they produce a given protein will continue to produce the protein when implanted into a laboratory animal. There is no reason whatsoever to think that similar cells implanted into a human (or any other animal) would behave any differently. Thus, Orkin et al.'s caveat about animal models of human genetic disease is irrelevant in the present context, and certainly does not suggest anything about unpredictability of the claimed invention.

Similarly, the Examiner's reliance on Orkin et al.'s statement concerning unpredictability of gene expression after *in vivo* transfer of genes into cells, and on the editorial's and Verma et al.'s comments concerning gene therapy, is misplaced. The present invention does not involve *in vivo* gene transfer. As discussed above, gene transfer in accordance with the invention is carried out *in vitro*, permitting selection, expression, and characterization of the transfected cells *in vitro*, prior to their use in therapy. Applicants fail to see what could be considered unpredictable about such a procedure. Even if stable transfection with a non-viral vector is a relatively rare event, doing it *in vitro*, where selection and expansion of appropriately transfected cells are routine, solves the unpredictability problems. The unpredictability issues discussed by Orkin et al., Verma et al., and the editorial do not apply to *in vitro* transfection. As the Examiner has cited no other publication that establishes the supposed unpredictability of the prior art pertaining to *in vitro* transfection of cells, this Wands factor must be deemed to support the patentability of the invention.

The quantity of experimentation.

The Office Action cites Orkin et al., the editorial, and Verma et al. as evidence that "a very large amount of experimentation of a complex nature will be required to develop any gene therapy protocol to the point of efficacy." (Office Action at page 3) As discussed above, none of the cited references is relevant to the present case, since none addresses *ex vivo*, non-viral methods of gene therapy.

While it may well be true that *in vivo* gene therapy will require extensive experimentation in order to achieve routine success, that is not true of the presently claimed *ex vivo* methods. *In vivo* gene therapy generally requires that DNA be administered directly to the patient. The DNA must then reach the target cells within the patient, be incorporated into an adequate number of target cells, and be expressed by the cells to a degree that is therapeutically effective. Furthermore, the vectors that seem to produce the best results with respect to those problems are viral vectors, which introduce their own problems revolving around safety. Finding ways around these problems with *in vivo* gene therapy may indeed require a large amount of experimentation. But even if that proves to be true, it is irrelevant to the present invention.

In contrast to *in vivo* gene therapy, the present method of *ex vivo* gene therapy permits manipulation of the cells *in vitro* to produce a final population of cells selected to ensure not only incorporation of the construct-derived DNA, but also uniform expression of the protein at a desired level. Thus, none of the issues relating to the direct administration of DNA to the patient (i.e., *in vivo* gene therapy) is pertinent to the present invention: the vagaries of *in vivo* gene therapy that may render it unpredictable simply do not apply in the present case.

The breadth of the claim

The Office Action at pages 3 and 4 states that

> The claimed methods, at the broadest, are drawn to treatment of any disease state with (presumably) any nucleic acid which would be useful in such treatment. Given the relative infancy, if not non-existence, of (successful) gene therapy, such represents relatively very large breadth of the claims.

Applicants note that, contrary to the Examiner's belief, the claims do not cover use of "any nucleic acid," but rather limit the constructs to those meeting the structural and functional limitations of the claims. These limitations define constructs that should work as described, i.e., should be capable of (1) being transfected into cells *in vitro*, (2) homologously recombining with genomic DNA at the site defined by the targeting sequence, and (3) directing expression of the endogenous gene from the exogenous regulatory sequence so that the cells express the desired protein. The Examiner has pointed to no evidence that would suggest any reason why one of ordinary skill would not expect the constructs to work in this manner. Nor is there evidence of record indicating why one of ordinary skill would believe the cells could not be successfully implanted in a mammal. To the contrary, Applicants have shown that *in vitro*-transfected cells producing a desired protein can be implanted and will continue to produce the protein within the animal. As with the hypodermic syringe analogy presented above, there is no rational basis for rejecting the present claims as overbroad just because they are not limited to a particular protein or a particular disease. The claims are broadly drawn because there is no reason to expect that the claimed method could not be used for any protein encoded by an endogenous gene of the vertebrate cell. The fact that *in vivo* gene therapy may not work is irrelevant.

The Office Action indicates on page 4 the belief that neither the specification nor the art provide sufficient guidance for one in the art to practice the claimed invention and that the

> practitioner would have been forced to turn to empirical experimentation to determine appropriate dosages, treatment regimens and other factors, required for successful practice of a gene therapy method.

Applicants again note that once the cells are transfected, selected, and expanded *in vitro* (all routine cell biology manipulations), it is a simple matter to quantify the amount of the therapeutic protein secreted by the cells and calculate the number of cells necessary to produce the desired amount. Implantation of cultured cells is yet another routine matter. Monitoring the amount of protein secreted into the bloodstream of the patient so that the dosage of cells can be adjusted as needed is also routine. Alternatively, of course, one could monitor the biological effect of the protein. Applicants fail to see any step involved in the claimed method that is likely

to require experimentation that rises to the level of "undue," or that is more difficult than that required for the development of any other type of pharmaceutical. Since, contrary to the Examiner's apparent assumption, the claims do not cover *in vivo* gene therapy, all of the uncertainty the Examiner associates with that field of endeavor is simply not material.

We note that the Office Action did not address the Wands factor related to the level of skill in the art. Applicants assert that the level of skill in the art of *in vitro* transfection is high, an evaluation that counts in their favor with respect to this type of analysis under § 112, paragraph 1.

Summary

In summary, none of the references cited in the Office Action provides any reason to believe that the presently claimed methods will not work as described. None of the specific problems discussed in these references applies to Applicants' methods. If anything, the enthusiasm these references express for gene therapy in general is consistent with the expectation that the present methods will work. Applicants submit that the Office Action does not provide any evidence that the present invention is not enabled.

Based on the arguments presented above, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, paragraph 1.

CONCLUSION

Attached is a marked-up version of the changes being made by the current amendment.

Applicants respectfully request that, in view of the arguments above, all claims be allowed. Enclosed is a check for $445 for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 07236-013004 referencing Attorney Docket No. 07236-013004.

Respectfully submitted,

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Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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Version with markings to show changes made

In the title:

TRANSKARYOTIC [PRODUCTION AND] DELIVERY OF [DNASE] THERAPEUTIC PRODUCTS

In the claims:

Claim 157 has been amended as follows:

157. The method of claim [497] 155, wherein the therapeutic product is human β-interferon.